

HTLV Type I (U. S. Isolate) and ATL (Japanese Isolate) Are the Same Species of Human Retrovirus

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Received October 3, 1983; accepted November 1, 1983

Two independent isolates of human leukemia virus, human T-cell leukemia virus (HTLV) and adult T-cell leukemia virus (ATLV), are shown to be the same by blotting analysis using gene-specific probes and restriction enzymes. Therefore, Japanese ATL virus and Caribbean HTLV type I, which are exogenous for human, have a common origin.

A human retrovirus, human T-cell leukemia virus (HTLV), was first reported by Gallo and his colleagues in 1980. It was isolated from a cell line established from a patient with cutaneous T-cell lymphoma (CTCL, mycosis fungoides) (1, 2), as well as from a patient with another type of CTCL, Sezary syndrome (3). Subsequently, adult T-cell leukemia virus (ATLV) was isolated from Japanese patients with adult T-cell leukemia (ATL) (4, 5), which is a new and unique clinical entity of T-cell malignancy (6), and ATL was shown to be closely associated with this unique T-cell leukemia, ATL (4, 5). In an early stage of studies, patients from whom HTLV was isolated were thought to have a disease different from Japanese ATL, because CTCL is a rare disease and only sporadic cases are known (7), whereas ATL shows clustering in southwest Japan (4-6, 8). Therefore, we thought that our viral isolate ATL was distinct from HTLV. In this paper, we report that these two independent isolates are the same retrovirus.

The relation between these two isolates, HTLV and ATL, seemed interesting and important for several reasons: (i) Complete nucleotide sequence analysis of the ATL genome showed a unique sequence (pX) that can code for extra protein(s), in addition to the ordinary genes, *gag*, *pol*, *env*, and two LTRs (9, 10). Therefore, this unique

pX region, which may have a biological function, could be different in ATL and HTLV. (ii) ATL and HTLV are endemic in completely different areas of the world, that is, the southwest part of Japan and the Caribbean (8, 11-14). Therefore, the problem of whether these viruses are identical is crucial in understanding the origin of the virus and the factors involved in viral transmission. (iii) The identities of these two viruses are important in evaluating the spectra of lymphoma or leukemia associated with these viruses. (iv) Recently, HTLV was suggested to be involved in acquired immune deficiency syndrome (AIDS) in the United States; however, no high frequency of AIDS or AIDS-like diseases has been reported in the ATL-endemic area in Japan so far.

HTLV and ATL were recently reported to be similar in immunological cross-reactivity (15, 16) and nucleic acid hybridization (17). Gallo and his colleagues (15, 16) demonstrated that sera from Japanese ATL patients reacted with HTLV proteins p19 and p24. Furthermore, viral cDNA prepared with purified HTLV and ATL virions showed similar cross-hybridizations with the two viral RNAs (17). These results were clear evidence that HTLV and ATL are similar, but were not sufficient to prove that the two viruses are identical, because the immunological cross-reactivities of p19 and p24 reflect only parts of the *gag* gene of the viral genomes, and it was also uncertain whether the viral cDNA prepara-

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tions were representative: at least, our ATL V cDNA used in previous work (17) was a copy of only the 5' end of the viral genome. Therefore, it was still possible that the two viral isolates were different species even if they had common sequences in part.

Therefore, we compared the proviruses of HTLV and ATL V, which are integrated in cells. The cell lines MT-1 and MT-2 containing ATL V (18, 19), and the cell line HUT102 containing HTLV (1, 20) were used. As the source of probes, a typical ATL V genome, a clone, λ ATK-1, isolated from fresh ATL leukemic cells (10) was used, because the nucleotide sequence of this provirus has been determined (10), and blotting analysis of 78 cases of ATL confirmed that this sequence is typical of ATL provirus (manuscript in preparation). From the clone λ ATK-1, the specific regions shown in Fig. 1 were subcloned in pBR322 and used as gene-specific probes.

Southern blotting analysis using *Eco*RI, which does not cut the provirus sequence (10), indicated that six, eight, and nine copies of proviruses are integrated in cell lines MT-1, MT-2, and HUT102, respectively (Fig. 2). Using gene-specific probes (Fig. 1), it was shown that a few copies were complete provirus genome, since they were detected with all the probes (data not shown). Therefore, the same viral DNA fragments should be formed by digestions with restriction enzymes of these cellular DNAs if they contain identical provirus. This possibility was tested by digestions with restriction endonucleases and then detection of viral sequences with gene-specific probes.

From the nucleotide sequence of ATL V provirus (10), *Pst*I should give three internal fragments of 1.3, 1.6, and 2.4 kbp

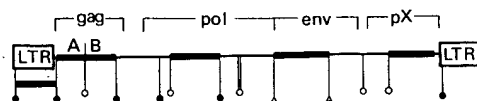


FIG. 1. Restriction endonuclease map of cloned ATL V (λ ATK-1). Boxes on the map represent fragments for specific probes, each of which was subcloned into pBR322. O, *Pst*I; ●, *Sma*I; Δ, *Bam*HI.

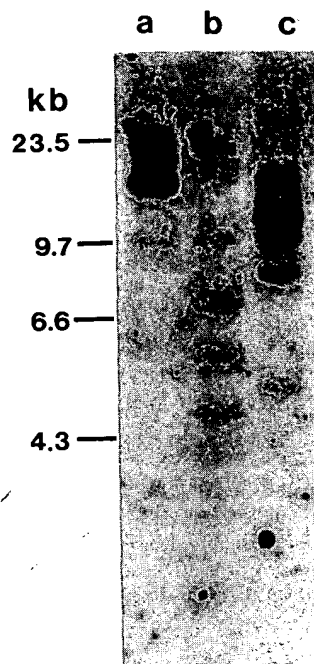


FIG. 2. Detection of provirus sequences integrated in cell lines. Samples of 10 μ g of each cellular DNA from cell lines MT-1 (a), MT-2 (b), and HUT102 (c) were digested with *Eco*RI and analyzed by the Southern blotting procedure with a 32 P-LTR probe.

(Fig. 1). With the *gag*-B-, *pol*-, and *env*-specific probes, ATL V and HTLV DNAs gave identical sizes for these fragments, indicating that the *gag-pol-env* regions of these viruses, which are covered by *Pst*I sites, are very similar (Fig. 3). Since the conditions used for hybridization are relatively stringent (0.6 M NaCl at 65°, then washing with 0.15 M NaCl at 65°), the sequences detected by this blot hybridization are identical or very similar. Less intense bands in some cases in Fig. 3 (for example, IV, lane b) reflect copy numbers of the integrated defective proviruses lacking a specific sequence, but do not reflect the inefficient hybridization due to sequence with low homology. Other bands were also detected, but these were shown to be derived from the defective provirus genomes integrated in the cellular DNA (data not shown) and thus did not affect this conclusion. Similarly, double digestions with

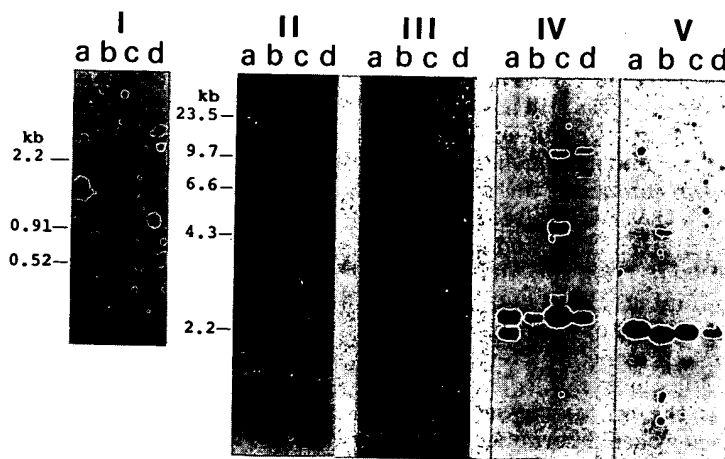


FIG. 3. Comparison of gene-specific fragments of the provirus DNA integrated in MT-1 (a), MT-2 (b), HUT102 (c), and ATK-1 clone (d). Restriction enzyme digests were analyzed by Southern blotting with hybridization at 65° in 0.6 M salt. Double digests with *Sma*I and *Pst*I were hybridized with *gag*-A probe (I); *Pst*I digests were hybridized with *gag*-B probe (II), with *pol* probe (III), and *env* probe (IV); and *Bam*HI and *Sam*I double digests were hybridized with *pX* probe (V).

*Sma*I and *Pst*I showed that the 5' parts of *gag* detected with *gag*-A-specific probe are the same (Fig. 3, I). Furthermore, the extra sequence *pX* was also shown to be very similar by *Bam*HI and *Sma*I digestions using a *pX*-specific probe (Fig. 3, V). In MT-2 DNA, the fragment containing the *pX* region was slightly smaller than those from other cell lines and this may be due to a small deletion or a new *Bam*HI site caused by a single base change in this region. This variation, however, does not affect the conclusion, because MT-1 and cloned provirus (λ ATK-1) from leukemic cell gave the same band as HUT102 cell. With the LTR probe, similarly efficient hybridizations and the same band sizes were detected (data not shown), suggesting that LTR sequences are also similar (9, 10). These observations clearly indicate that the locations of the gene-specific sequences and the cleavage sites of some restriction enzymes are identical on the proviral genomes integrated in MT-1, MT-2, λ ATK-1 clone, and HUT102. Thus, it is concluded that HTLV and ATL are the same species of retrovirus, even if they differ in base replacements or very small insertions or deletions. This conclusion does not exclude

very small systematic sequence variations between two viral isolates, although it is unlikely. The identity of HTLV and ATL suggests that the viral populations in southwest Japan and the Caribbean have a common origin. Very recently, Wang-Staal *et al.* reported that several HTLV isolates from various places in the world are similar, identifying only *pol-env* fragments by blot hybridization (21). Their finding on the *pol-env* region is in agreement with our result as confirmed in Fig. 3, III.

The finding that HTLV and ATL are the same is compatible with recent pathological and clinical studies which suggest that HTLV-associated CTCL in the United States and Europe is the same disease as Japanese ATL (11, 12, 14), and the findings also suggest the common origin of the viruses localized in Kyushu, Japan, and the Caribbean.

Since two isolates are the same species of retrovirus, the different terminologies are confusing. We will use the term HTLV for our isolate too; for example, the provirus contained in a clone, λ ATK-1, which was sequenced completely, is now called HTLV_{ATK}.

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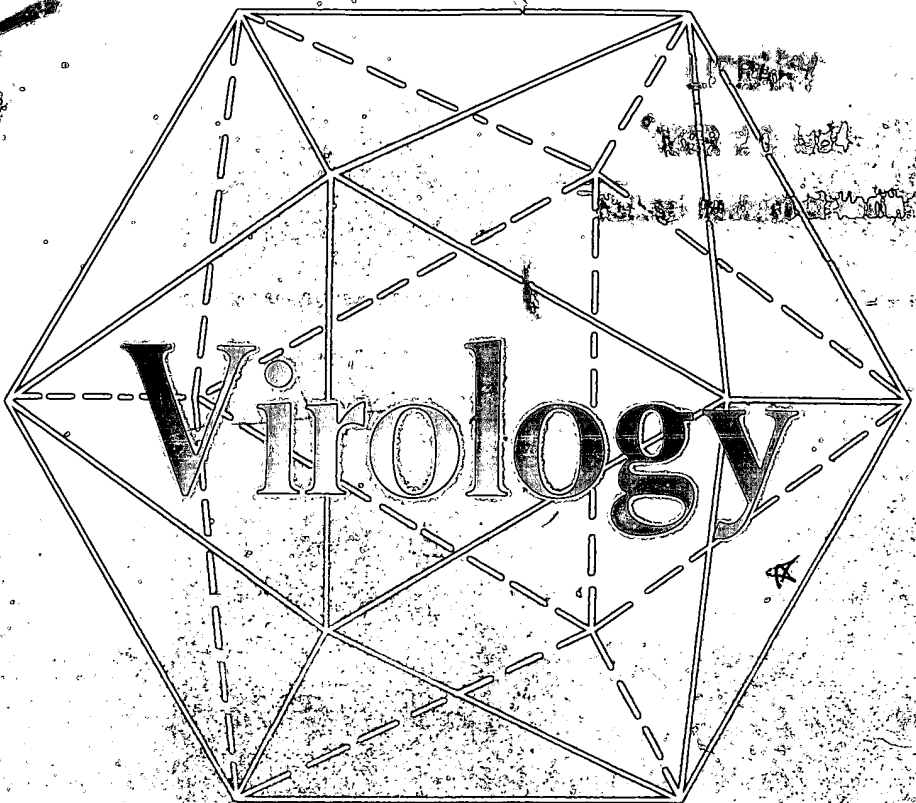
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ACKNOWLEDGMENTS

The authors thank Dr. R. C. Gallo and Dr. I. Miyoshi for generous gifts of cell lines, Dr. S. Hattori for valuable discussion, and also Ms. Y. Hirayama for technical assistance. This work was partly supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan.

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